ZOOLOGICAL SCIENCE 10: 873–885 (1993)

REVIEW

Control Strategies of Thyroid Hormone Monodeiodination in Vertebrates

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INTRODUCTION

In all vertebrates thyroxine (T_4) is the main or even exclusive secretory product of the thyroid gland. Mammals may differ since in rats [31], dogs [110], mice [111] and even man [49] thyrotropin (TSH) stimulates the intrathyroidal conversion of T_4 to T_3. This may lead to a release of T_3 from the thyroid prior to T_4 following stimulation by TSH or TRH as has been shown to occur in sheep [86]. Nevertheless, it has been calculated that more than 80% of the body's T_3 and rT_3 is derived from peripheral deiodination of T_4 [9, 30].

In non-mammalian vertebrates an injection or release of TSH increases plasma concentrations of T_4 without a detectable influence on circulating levels of triiodothyronine (T_3). This has been shown in fish [61], amphibians [50], and adult chickens [58, 60]. In quail [81] and chick embryos [58] TSH increases plasma concentrations of both T_3 and T_4. In quail there is little change in the plasma T_3 to T_4 ratio after TSH stimulation, whereas in the chicken this ratio is decreased. Consequently virtually all circulating levels of T_3 and of reverse T_3 (rT_3), if detectable, are the result of deiodination reactions occuring in peripheral tissues.

Degradation of T_4 is primarily by deiodination, but a lesser fraction may undergo conjugation, side-chain deamination or decarboxylation, and ether bond cleavage [9, 73]. Monodeiodination of the phenolic ring of T_4 is called the outer ring deiodination (ORD) (5′-monodeiodination (5′D)). By this reaction T_4 is converted to 3,3′,5-triiodothyronine (T_3) and 3,3′,5′-triiodothyronine (rT_3) to 3,3′-T_2. The other reaction is the monodeiodination of the tyrosyl ring, called the inner ring deiodination (IRD) (5-monodeiodination (5D)). IRD converts T_4 to rT_3 and T_3 to 3,3′-T_2 (Fig. 1). Since T_3 is considered to be the only active thyroid hormone, the ORD reaction may be regarded the activating pathway of T_4 metabolism as it produces T_3, while the IRD reaction is considered to be the inactivating pathway as it produces the inactive rT_3 and degrades the active T_3. Since the relative rates of ORD or IRD appear to determine the ultimate biological effect of the T_4 secreted by the thyroid gland, it should be clear that any factor, endogenous or exogenous, influencing this balance, is very important for thyroid hormone formation and activity.

T_3 exerts its biological effect by binding to nuclear binding sites [85] and may originate from the intracellular T_4 to T_3 conversion or be derived from the plasma. Local T_3 production is important in the anterior pituitary and the brain, whereas in other tissues e.g. the liver, kidney and heart the T_3, derived from blood plasma, occupies about 75% of the bound nuclear binding sites, while the receptors of these tissues are 50% saturated [100]. However, these hormone importing tissues are usually hormone exporting ones as well.

In the following review a comparison with the mammalian model is made of the different deiodinase types as found presently in vertebrates. Since the activity of these enzymes may vary consider-

Received April 26, 1993

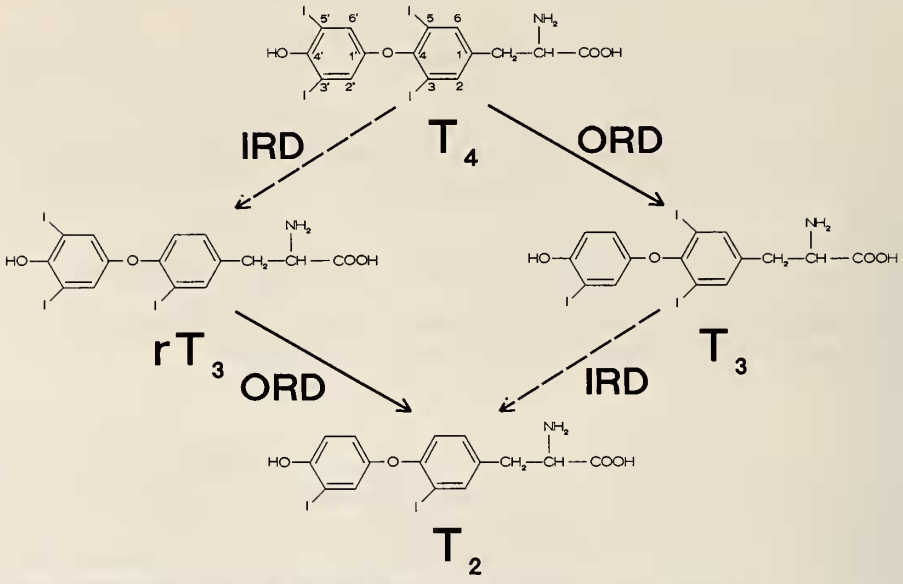


Fig. 1. Sequential outer ring deiodination (ORD) and inner ring deiodination (IRD) of T_4 to T_2.

ably during development, these data as far as available are included also (see also McNabb [79]).

THE MAMMALIAN MODEL

a. *The different deiodinase types of mammals*

Three different iodothyronine deiodinating enzymes have been distinguished, according to the selectivity of the reactions they catalyze, their substrate preference and their susceptibility to inhibition by 6-propyl-2-thiouracil (PTU) [55]. The most important features of the different types, based mainly on studies in rats, are summarized in Table 1.

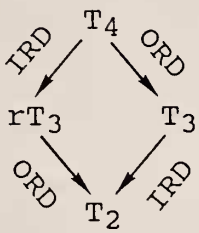
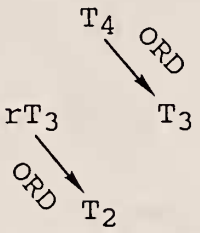
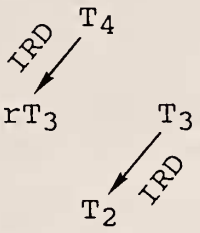
The biochemistry of these three deiodinases has been comprehensively reviewed by Leonard and Visser [73]. Since then, considerable effort has been put in the purification of these enzyme types. Up till now, all reports deal with the isolation of one protein, with characteristics corresponding to the type I enzyme. No progress is published concerning the purification of the other empirically defined deiodinase types, but this could of course be linked to the fact that these enzymes are only present in low amounts compared to the type I enzyme.

It is proven that during subcellular fractionation, the activity of all three types of deiodinase is associated with the crude microsomal (Mx) fraction, including all membrane fractions. To express activity, however, they all need the presence of a cytosolic cofactor. The exact nature of this cofactor remains uncertain, but the importance of sulfhydryl groups seems to be generally accepted. Some studies even suggest that the intracellular ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) could be the intracellular modulator of the ORD activity *in vivo* [43]. Several reduced thiols (RSH) have been shown to stimulate the deiodination reactions *in vivo* and *in vitro*, and the cofactor most oftenly used *in vitro* is the thiol reducing agent dithiothreitol (DTT).

b. *Evaluation of the enzyme assays*

Since the most important biochemical properties of the 3 mammalian enzyme types are presently known, it is possible to develop specific standardized tests for each enzyme type. This greatly facilitates the comparison of results from different research groups, something that proves to be difficult for many of the earlier results because a great variety of tests have been used and it is not always clear which enzyme type(s) interfere in the assay.

TABLE 1. Characteristics of the different deiodinating enzymes in mammals; ORD=outer ring deiodination; IRD=inner ring deiodination

	Type I	Type II	Type III
			
Main tissue localization	liver kidney thyroid	CNS pituitary BAT placenta	CNS placenta skin fetal intestine
Substrate preference	$rT_3 \gg T_4 > T_3$	$T_4 > rT_3$	$T_3 > T_4$
K_m	T_4: 2 μM T_3: 5–10 μM rT_3: 0.1 μM	T_4: 1 nM rT_3: 2 nM	T_4: 50 nM T_3: 10 nM
Thiol requirement	3 mM	20 mM	10 mM
PTU	inhibitory	no or little effect	no or little effect

When comparison has to be made with deiodination studies in non-mammalian vertebrates even more difficulties arise. Only in very few species attempts have been made to characterize in detail the biochemical nature of the enzymatic activity measured although this is essential for correct interpretation of the results. A good example for this is the *in vitro* test on liver (or other tissue) homogenates where the amount of T_3 is measured after incubation with T_4 as substrate. This test has been widely used for measuring the so-called T_3 producing activity by ORD (5'D). However, detailed studies showed that, especially in tissues where also type III activity is present, the T_3 recovery in this test was influenced both by T_3 producing (ORD) and T_3 degrading (IRD) activity [24]. The use of low substrate and high enzyme concentration even increased the relative impact of IRD in the "5'D" test and therefore it cannot be regarded as a reliable ORD test. From this it can be concluded that it is very important to choose a test procedure that is specific for the enzyme type to be measured and that results from non-standardized assay procedures must always be compared with great care.

c. *Evolution of deiodinases during development*

The placenta of several mammals studied have type III deiodinating activity which may serve as a barrier to prevent trans-placental passage of T_4 and T_3 from the mother to the fetal serum. This may account for the majority of the amniotic fluid and serum rT_3 and the observed peak in rats at gestation day 16 of both placental type III and amniotic fluid rT_3 [18, 93]. Normal fetal serum levels of T_3 are low and of rT_3 high whereas T_4 concentrations rise progressively to the end of gestation. Immediately after (lam, infant) birth a dramatic rise in T_3 occurs independently from the simultaneous surge in serum TSH indicating a rapid activation of T_4 ORD. The altricial rat is somewhat different in this regard since neuroendocrine control of thyroid embryogenesis is only completed after parturition and maximal T_3 levels will not be obtained before 4 weeks of age [32]. Rat and human placentae also can convert T_4 to T_3 by type II activity, which may be important to maintain normal placental and/or decidual function. In fetal sheep type I and type II pathways are described in BAT suggesting that type I may be

important for T_3 supply to the plasma while type II has a function in thermogenesis [56].

Despite low circulating levels of T_3 during development in mammals, the presence of type II ORD activity in brain and hypophysis may be important for intra-organ T_3 production and the development of the central nervous system. Support for this comes from the observation that hypothyroidism in fetal and neonatal rats increases type II ORD in these tissues and BAT [56].

d. *Influence of growth hormone (GH) on deiodinase activity in mammals*

A decrease in plasma T_4 and an increase in plasma T_3 occur in patients with pituitary idiopathic dwarfism receiving human GH [34, 91, 96]. In the newborn lamb ovine GH (oGH) but not ovine prolactin (oPRL) may increase the plasma concentrations of T_3 and to a minor extend free T_4 whereas rT_3 decreases [63]. High levels of GH have been reported in blood of fetal lambs and these levels decrease after birth, despite continued postnatal growth [1]. These observations suggest that GH may be a candidate for the activation of thyroid hormones by increasing the transformation of T_4 to T_3 and decreasing the formation of rT_3.

In fed dwarf goats oGH raised plasma concentration of T_3 without affecting T_4. This increase was more pronounced when goats were food deprived for 7 days. Here at the same time the hepatic, but not the kidney, ORD activity (measured as the T_3 recovery from tissue homogenates incubated with T_4, see evolution of assays) was stimulated following the GH injection [48]. Interestingly, in cows bovine somatotrophin (BST) increased milk production and hepatic DNA without altered serum concentrations of T_4 or T_3. ORD-activity (T_3 recovery from T_4) was unaffected in homogenates of liver and kidney but increased about twofold in mammary tissue in response to BST [15]. Following prolonged BST treatment however increased ORD-activity was found in kidney and liver [95], but again the *in vitro* method used can not distinguish between an increased T_4 into T_3 conversion (ORD) or inhibition of T_3 degradation (IRD).

In rats plasma concentrations of thyroid hormones (TH) were not influenced following GH injections, but hepatic 5'D activity (T_3 recovery following T_4 administration) was increased [62]. Observations on rats have also demonstrated that a continuous infusion of GH at a dose of 120 μg/day throughout 3 weeks is capable of increasing the amount of locally produced T_3 in the liver, whereas plasma concentrations of T_3 and T_4 remain the same as in controls [42]. More recently the effect of GH and T_4 substitution on liver deiodinase type I activity was checked in hypophysectomized rats. Hypophysectomy severely depressed this activity and GH substitution alone significantly increased this activity. When T_4 was administered alone ORD-I was normalized, but GH together with T_4 administrations resulted in a rather depressing effect indicating a modulating role for GH of the T_4 effect on this specific enzyme activity [6].

DEIODINATION IN BIRDS

a. *The presence of deiodinases*

Chickens, Japanese quail and ring doves are the birds most often used in avian deiodination studies. Nearly all of the kinetic studies on ORD and IRD activity hav been performed on liver tissue, either on whole tissue homogenates or crude microsomal fractions (Table 2). The presence of a type I enzyme has been documented most extensively in chicken and quail liver [37, 78], although ORD has also been described in kidney [23, 78]. Contrary to the situation in mammals considerable type III activity was demonstrated in chicken liver [7, 20, 22, 24, 37, 46]. However no ORD-II like activity could be found.

In chicken brain, both type II and type III-like activities have been found (Rudas, personal communication). All these results taken together lead to the assumption that the deiodinase system of birds is very much like that in mammals [79].

b. *Ontogeny of deiodinase activity*

In the chick embryo the hypothalamo-adenohypophyseal-thyroid axis appears to be functional at about days 10–13 of development [104]. Plasma concentrations of T_4 increase during embryonic life and reach a maximum at day 20 of incubation. Plasma levels of T_3 are however low

TABLE 2. Characteristics of iodothyronine deiodinases in avian hepatic tisuue. Measurements are made on homogenates (1) or microsomes (2)

Species	Activity	K_m	Vmax pmol produced/ mg prot/min	PTU inhibition	Reference
chicken (1)	ORD	T_4: 1.16 μM	45 T_3	yes	Lam & Harvey [67]
chicken (1)	ORD-I	T_4: 0.57 μM	102 T_3	yes	Rudas [94]
		rT_3: 0.12 μM	214 I^-		
quail (1)	total ORD	T_4: 1.58 μM	0.029 T_3	part	McNabb *et al.* [80]
chick embryo (2)	ORD-I	rT_3: 0.14–0.18 μM	200–650 T_2	yes	Galton & Hiebert [37]
	IRD-III	T_3: 2.91–3.96 nM	0.20–2.68 T_2	no	
ring dove (1)	ORD-I	rT_3: 0.44 μM	255 rT_3 degr.	yes	Rieman & McNabb [92]

up to day 19 of incubation and increase during pipping and hatching. Embryos that have perforated the air space membrane on the day prior to pipping have increased T_3 levels, whereas T_4 does not change. The consequent sharp increase in T_3/T_4 ratio following the transition of an allantoic into a pulmonary respiration suggests that T_3 has an important role in the proces of pipping and hatching [59].

The hepatic type I activity showed a 3-fold increase in the chick embryo up to the period of pipping and hatching and decreased thereafter. Hepatic type III activity increased 3-fold between incubation day 14 and 17, but decreased more than 10-fold to almost basal levels which persisted posthatching [7, 24, 37, 64, 105] (Fig. 2). The decrease in type I activity observed in posthatch chicks is comparable to results on total liver ORD in posthatch quail using the T_3 recovery method [46].

The changes found in these precocial birds are clearly different from the ontogenic pattern of deiodinases in mammalian liver. In the altricial rat 5'D-I activity increases during the last week of gestation and continues to increase after birth, while IRD-III activity is low and comparable in fetal and adult liver [40, 45]. In sheep too, kinetic studies show that the ratio of mean production rate of rT_3/T_4 (IRD) is similar in fetal and maternal sheep, while the ratio of mean production rate of T_3/T_4 (ORD) is much lower in the foetus [17]. However, although type III activity seems not important in mammalian fetal liver, substantial activity is found in the placenta, where it decreases during the last days prior to parturition [93].

d. *Influence of GH on deiodinase activity in the chicken*

Several studies have revealed that GH is responsible for the dramatic changes in TH which occur in the chick embryo at the end of incubation. So, an injection of total GH into chick embryo [3, 21], the glycosylated variant of cGH [2] or hypothalamic hormones which release GH [65] will stimulate T_3 levels in circulation. This effect however is no longer present in normally fed chicks 2 days after hatching [23].

The *in vivo* increase in T_3 can be linked to an increased ORD activity measured as *in vitro* T_3 recovery from liver homogenates incubated with T_4 [23, 58]. Specific type I and type III deiodinase tests, however, show that GH has no effect at all on the amount of hepatic type I enzyme (catalyzing T_4 deiodination to T_3) but acutely decreases the activity of the type III enzyme (catalyzing T_3 deiodination). This suggests that the GH-induced increase in plasma T_3 is not due to an increased T_3 production, but is the result of a decreased T_3 breakdown. The lack of stimulatory effect of GH injection in 3-day-old fed chicks might be the combined result of a low hepatic type III enzyme level and a low GH receptor availability at that stage [20, 24, 107] (Fig. 3).

Since posthatch chicks lack the ability to increase plasma concentrations of T_3 after GH injection, one can question the importance of GH in maintaining plasma levels of T_3 during growth in the chicken. The IRD-III activity is however very low during growth, indicating that endogenous GH

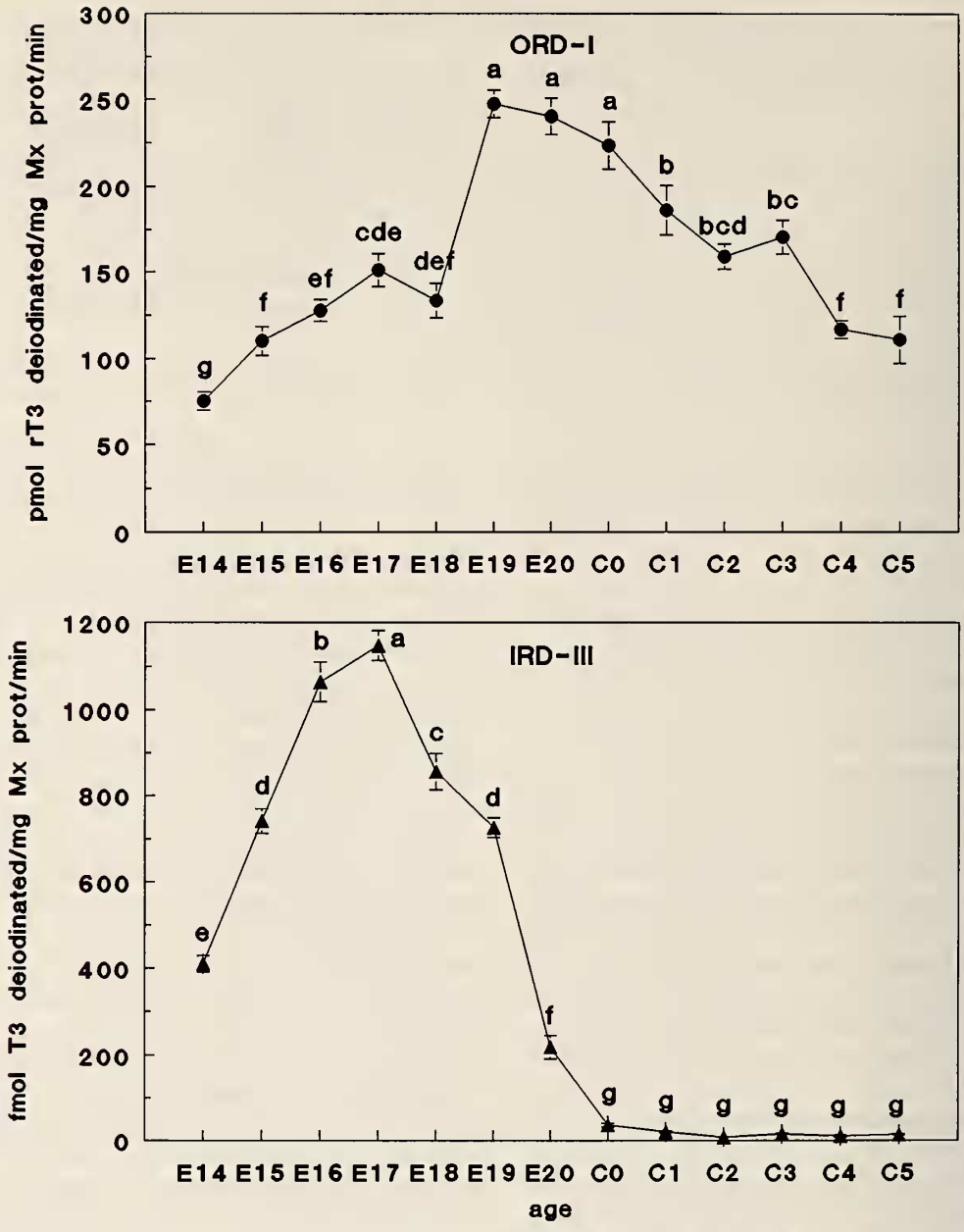


Fig. 2. Ontogeny of ORD-I and IRD-III activity in the liver of chickens during the last week of embryonic development and the first week posthatch. Values shown are mean ± SEM for five different pools. Means with the same letter are not significantly different (ANOVA, $P<0.05$) (Darras *et al.* [24]).

may exert already a maximal inhibitory effect on T_3 degradation. This has been shown by hypophysectomy which clearly increases IRD-III, whereas injection of GH counteracts this increase [22]. Moreover, an injection of anti-GH into 4-week-old chicks decreases plasma concentrations of T_3 compared to controls. These observation confirm that the regulation of hepatic type III, but not of type I deiodinase, is GH dependent.

The question arises which factor might interfere

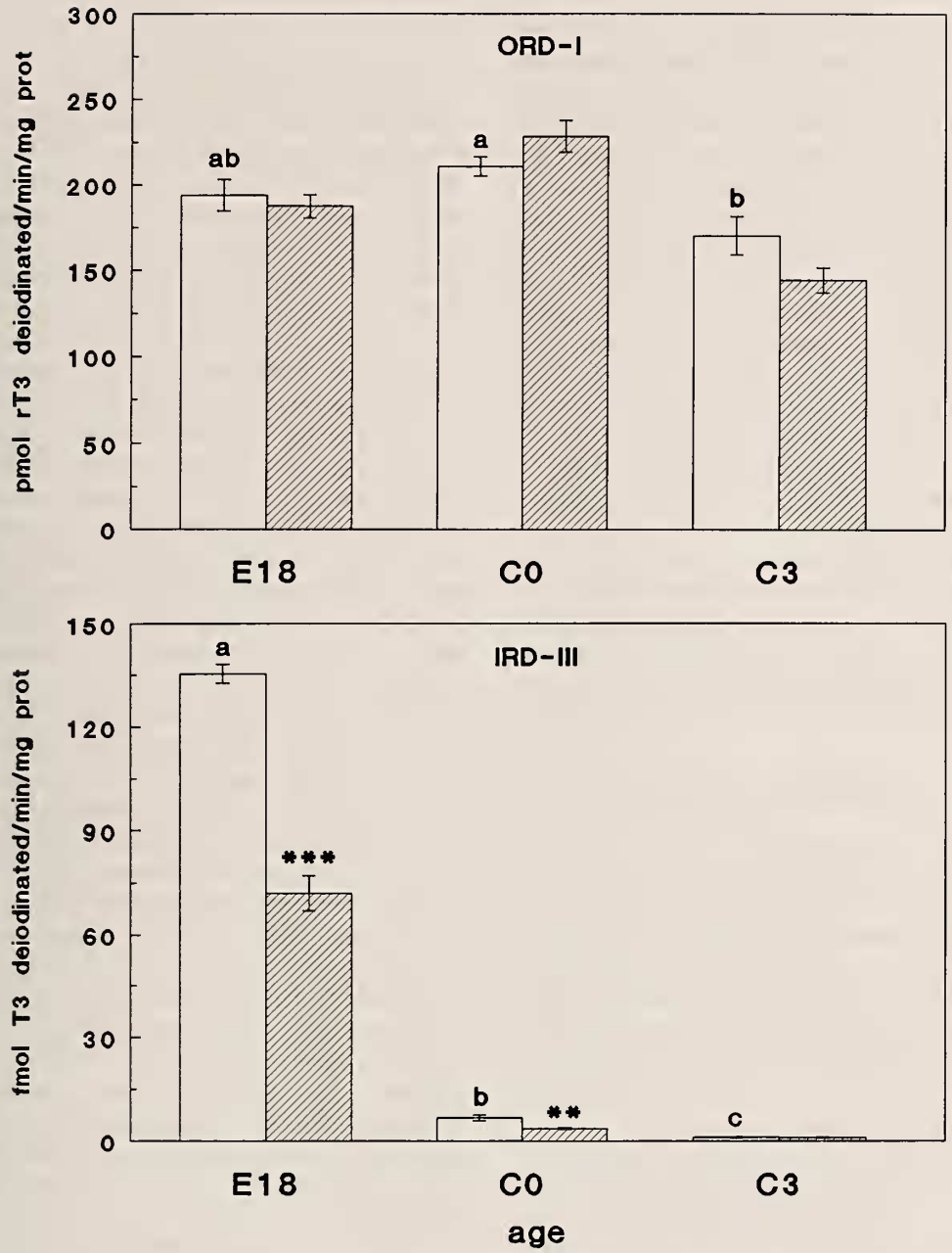


FIG. 3. ORD-I and IRD-III activity in hepatic microsomal fractions of embryonic and pothatch chicks 2 h after injection of saline or 20 μg chicken GH/animal. Means $\pm$ SEM for 10 different pools/group. $^{**}P<0.01$, $^{***}P<0.001$ compared to control groups of the same age (ANOVA) (Darras *et al.* [20]).

with the control of ORD-I and hence the peripheral production of T_3. In growing chicks T_4 levels are maintained up to 1 week after hypophysectomy whereas ORD-I slightly decreased after 3 days, but did no differ from sham operated controls after 1 week [22]. Also, a positive correlation was found between plasma concentrations of T_4 and the hepatic type I activity during ontogeny [24]. In

rats, this stimulatory effect of T_4 on ORD-I was known for sometime [54] and recently it has indeed been shown that thyroid status regulates the transcription of type I mRNA in rat liver microsomes [5]. Hypophysectomy notoriously decreased GH plasma concentrations but increased dramatically the number of GH receptors in the chicken liver [106]. This way the higher level of IRD-III activity will make an effect of GH more easily detectable since the higher availability of receptor sites further increases the effectiveness of the injected GH.

In the adult chicken, an injection of GH or TRH increases plasma concentrations of T_3, whereas T_4 even might be decreased [58, 60, 63, 66]. Although ORD-I activity is present in hepatic microsomal fractions, though less than in growing chickens, an injection of GH does not affect the amount of this enzyme and therefore does not stimulate the *in vitro* conversion of T_4 into T_3, as previously postulated. Instead GH decreases IRD-III and inhibits therefore T_3 degradation in adults as it does in embryos and growing chicks [20].

DEIODINASES IN REPTILES

Extrathyroidal conversion of T_4 to T_3 has been shown in a lizard (*Calotes versicolor*) after *in vivo* T_4 injections. This conversion can be inhibited by IOP administration [16, 57]. *In vitro* characterization of hepatic ORD activity has been performed on another lizard, *Sceloporus occidentalis,* revealing the presence of a high K_m enzyme inhibited by PTU, comparable to the mammalian and avian ORD-I enzyme [53].

PERIPHERAL PRODUCTION OF T_3 AND rT_3 IN AMPHIBIANS

Plasma concentrations of thyroid hormones are low during the life cycle of all amphibians studied and thyroid hormone action in postmetamorphic animals is poorly understood. Only during climax a profound increase in TH occurs, which appears to be indispensable for the induction of metamorphosis. Complete metamorphosis can be induced with T_3, T_4 or TSH, which is known to increase plasma concentrations of T_4, but not of T_3.

a. *Deiodinases during metamorphosis*

The occurrence of T_4 ORD activity in amphibians was first suggested in anuran tadpoles [71], mainly based on the plasma T_3 surge during climax, which may even surpass T_4, and the constantly very low T_3/T_4 ratio in the thyroids throughout metamorphosis, in *Xenopus laevis* [71, 98], and in *Rana catesbeiana* [101]. Confirmation was obtained when injection of ^{125}I-T_4 into transforming tadpoles of *Xenopus laevis* [72] and *Rana catesbeiana* [41] revealed peripheral production of ^{125}I-T_3. This ORD is thyroid hormone dependent and reaches maximal activity at midclimax [11]. The conversion of T_4 into T_3 appears to be a fundamental step in thyroid hormone induction of metamorphosis. Delayed metamorphosis together with a significant reduction in peripheral T_3 production are obtained with inhibitors of this ORD activity [10, 36]. By inhibiting the ORD activity and IRD activity, both present in premetamorphic bullfrogs, using iopanoic acid (IOP) and subsequent exposure to T_3, increased leg length and hepatic carbamyl phosphate synthetase activity, two indices of metamorphosis, could be obtained. These indices however were inhibited after exposure to T_4 indicating the importance of a T_4 to T_3 conversion during metamorphosis [36].

A detailed study of different tadpole tissues indicates that the ORD system exhibits K_m values for T_4 and rT_3 in the nM range and that T_4 is the preferred substrate. This activity is also unaffected by 1 mM PTU in the presence of 10 mM DTT and all these properties are comparable with the mammalian ORD-II enzyme [38]. The partial inhibition of ORD by PTU in the presence of low DTT concentrations as observed in *Rana catesbeiana* [38] and *Rana ridibunda* [51] tadpoles is not really in contrast with the characteristic of a type II enzyme. Such an inhibition has also been reported by Goswami and Rosenberg [44] for ORD-II in rat brown adipose tissue.

Generation of T_3 from T_4 *in vivo* in *Rana catesbeiana* tadpoles was not demonstrable until just before either spontaneous or induced metamorphic climax [39]. This could be due to the absence of T_3 generating activity or prevention of

T_3 accumulation by the presence of IRD-III. During premetamorphosis, IRD-III activity with a substrate preference for T_3 and a K_m value of 3.7 nM was found in liver gut and kidney. This activity was not inhibited by 1 mM PTU in the presence of 20 mM DTT and therefore relates the amphibian IRD enzyme to the mammalian IRD-III. At the same time ORD activity was undetectable during premetamorphosis in liver, tail, heart and kidney, minimal in brain and gut and could be quantitated only in skin. At mid climax this activity was increased more than 5-fold in skin and gut with tail tissue also acquiring ORD activity when tail resorption commences [39].

The situation in premetamorphic tadpoles therefore resembles the one in embryonic chickens with the presence of high 5D-III activity that declines rapidly at the end of incubation reaching minimal levels posthatching [24]. This way high concentrations of T_3 are obtained at metamorphosis or prior to hatching by an increase in T_3-generating activity together with a profound decrease in T_3-degrading activity.

In the neotenic axolotl evidence for the role of peripheral ORD activity is equivocal. In neotenic axolotls the thyroid T_3/T_4 ratio ($2.41 \pm 0.18\%$ ($n=12$)) did not differ from content ratio in metamorphosed animals ($2.01 \pm 0.23\%$ ($n=13$)), whereas the plasma ratio was significantly elevated in both conditions (8.7 ± 0.7 ($n=12$) and 16.3 ± 1.8 ($n=13$) respectively). The higher plasma ratio in metamorphosed animals may indicate a more pronounced peripheral T_3 generating activity following metamorphosis. However ORD activity as measured *in vitro* by the production of T_3 following addition of unlabelled T_4 could not be detected in liver or kidney homogenates from either neotenic or metamorphosed axolotls [52].

b. *Adult amphibians*

The existence of ORD activity in adult frogs has been demonstrated by the peripheral *in vivo* production of ^{125}I-T_3 from ^{125}I-T_4 in *Rana catesbeiana* [35], *Xenopus laevis* [11] and *Necturus maculosus* [35]. Following injection of unlabelled T_4 and consequent T_3 radioimmunoassay this activity was also detected in *Bufo marinus* [97].

Using the T_3 generating capacity as measured by RIA of tissue homogenates following addition of unlabelled T_4 as an index for ORD activity it was found that in *Rana ridibunda* the kidney and not the liver exhibited considerable activity [51, 108] with a significant annual variation. This T_3 production was very high in April, May and June and was followed by a minimum in July and August. This T_4 to T_3 conversion could also be detected in skin homogenates prepared from these frogs. Using an alternative approach of this *in vitro* technique by incubating tissue homogenates with radioactive substrates (rT_3 or T_4 for ORD and T_3 for IRD) followed by chromatographical separation of the resulting products, both ORD and IRD-III could be monitored in *Rana catesbeiana*. IRD-III activity was detected in liver, kidney, heart, intestine, brain, muscle and skin. In contrast, ORD activity was not detected in any of these tissues except gut and skin as in the tadpole [39]. Other investigators, however, suggested that in the kidney of adult frogs ORD activity may be present with preference for T_4 as substrate and therefore resembling the mammalian type II enzyme [51].

DEIODINATION IN FISH

a. *Deiodinases present in fish*

After an initial failure to measure deiodinase activity in liver of *Salvelinus fontinalis* [68], probably due to the absence of thiol factors and the insensitivity of the detection method, deiodination was mainly studied *in vivo*, following the kinetics of ^{125}I in plasma of fish injected with ^{125}I-T_4 [26]. Later deiodination activity was quantified after incubation with T_4 as substrate by measuring the T_3 recovery in tissue homogenates by radioimmunoassay [69] or by measuring the amount of radioiodide produced after separation on G-25 Sephadex columns [87]. Only later the dependence on thiol factors was evidenced [29] and more precise results were obtained using microsomal fractions instead of homogenates [99]. Salmonids and the eel were mostly used for T_4 deiodination studies in fish, but Leatherland *et al.* [70] surveyed thirty-three species from different teleostean families.

Deiodination activity is mainly found in liver

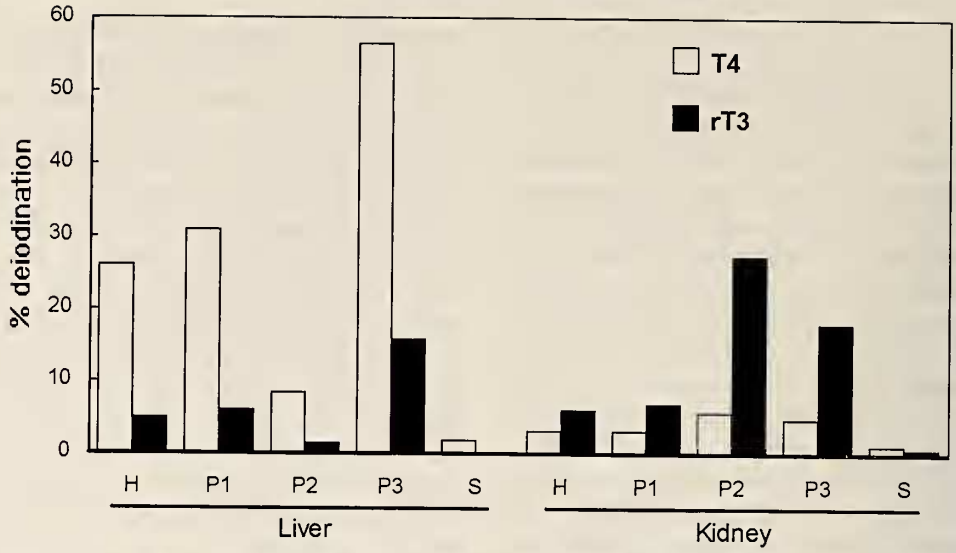


Fig. 4. Deiodination rate in different subcellular fractions of liver and kidney of *Oreochromis niloticus* after incubation of 1 mg protein with T_4 and rT_3. H=homogenate; P1=25,000×g pellet; P2=100,000×g pellet; P3=100,000×g upper layer; S=10,000×g supernatant.

and kidney [14, 69, 76] although other tissues, like gill and muscle display T_3-producing activity too. Far less is known about the exact nature of the deiodinases present in fish. Until now a detailed characterization of different types of enzymes, similar to the mammalian types, is only done in tilapia [84], where the liver ORD resembles the mammalian type II and the kidney enzyme has similar characteristics as the mammalian type I, but is insensitive to PTU and other seleno-protein inhibitors (Fig. 4). In other species a comparison with the mammalian model is difficult since only T_4 is used as substrate and only PTU is used as enzymatic inhibitor. This way a low K_m-enzyme is found in trout liver together with a high K_m-enzyme, which is also present in kidney [76].

b. *Ontogeny of deiodination*

In tilapia, monodeiodination of T_4 into T_3 is very low in fertilized eggs and larvae until 5 days after hatching and afterwards it increases until 30 days after hatching [90]. No further data are present for the ontogeny of deiodinases in fish.

c. *Hormonal regulation of deiodinases*

Extensive evidence exists for three groups of hormones affecting deiodinases in fish: e.g. growth hormone, cortisol and gonadal steroids.

In eel (*Anguilla anguilla*) GH from various vertebrates increases the conversion of T_4 to T_3 [25]. In rainbow trout (*Oncorhynchus mykiss*) similar results were obtained [75, 77], although recently Sweeting and Eales [103] demonstrated that GH has no direct effect on the deiodinase activity in cultured hepatocytes. Totally contradictory results were obtained in tilapia, where somatostatin seems to increase T_3 production during hyperthyroxinemia [13, 14].

In general, a decrease in plasma T_3 levels is observed following cortisol treatment due to an increased clearance rate from the blood [88, 89]. In *Salvelinus fontinalis* [109] and in *Cyprinus carpio* [4] cortisol stimulates the *in vitro* conversion of T_4 into T_3, while in *Oncorhynchus mykiss* no effect was found [8]. Testosterone and other male steroids [74] or their derivates have stimulatory effects on the conversion of T_4 into T_3 [47]. High doses of 17β-estradiol, which are present during vitellogenesis, inhibit monodeiodination of T_4 into T_3 [19], while low doses seem to have a stimulatory effect [33].

When plasma concentrations of T_4 are elevated following a TSH, porcine FSH or ovine LH challenge, little or no changes in plasma T_3 levels can

be observed [12, 82, 83]. It seems, however, that during hyperthyroxinemia at least in the tilapia, deiodination pathways try to restore normal T_4 levels by degrading T_4 into rT_3 [12]. An induced excess of T_3 on the other hand leads to production of rT_3 together with an inhibition of T_4 deiodination [27, 28, 102].

SUMMARY

In all vertebrates the main secretory product of the thyroid gland is thyroxine which may be considered as a relatively inactive prohormone to be used as a substrate for deiodination processes in peripheral tissues. Activation and inactivation of T_4 occurs in the presence of outer ring (ORD) and inner ring deiodinases (IRD), respectively.

Mammalian type I deiodinase is found in birds only, whereas in cold blooded animals no clear evidence for the presence of exactly the same ORD-I is existing. Several ORD and IRD activities have been described for amphibians and fish with different biochemical characteristics.

The tissue localisation of these deiodinase types differs profoundly among vertebrates.

During ontogenesis high plasma levels of T_3 occur at parturition or hatching in precocial mammals and birds, and at metamorphic climax of amphibians. Evidence is existing that these high levels may be obtained by an increased ORD-I or ORD-II activity but also by a decreased IRD-III activity resulting in an inhibition of T_3 degradation.

In mammals, birds and fish GH has been described as a stimulatory agent for ORD-activity. At least in the chick embryo, GH does not increase the amount of ORD-I but inhibits IRD-III activity and by doing so T_3 degradation.

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